A new approach to the study of bivalve evolution

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Abstract. Systematic and evolutionary studies of the Bivalvia have been based mostly on obvious conchological characters, but such characters could often reflect parallel adaptations and not phylogenetic relationships. Of the various biomolecular techniques capable of measuring genetic relationships among taxa, radio-immuno-assay (RIA) is particularly suited for bivalve studies. RIA measures genetic distance between taxa by measuring how much antibody made against proteins of one species binds with proteins from another. The results correlate highly with DNA hybridization, DNA sequencing and microcomplement results. RIA is unique in that it can extract information from proteins preserved in fossil and recent calcareous matrices. Because most molluscan collections consist predominantly of dry shells, RIA could prove to be very important for their evolutionary studies. I am using RIA to study parallel evolution in Veneridae and to develop a phylogenetic outline of the family. Venerid taxonomy is based currently on obvious conchological similarities. Preliminary results indicate that: 1) obvious conchological similarities can be parallel adaptations; 2) closely related species could exhibit wide conchological divergences in response to different life strategies; 3) Veneridae is deeply divided, but its origin is monophyletic. More accurate classifications could need to depend more strongly on anatomical, biomolecular and biogeographical data.

Bivalve systematics and studies of bivalve evolution have been based historically upon conchological characters because mollusc collections, both recent and fossil, consist primarily of dry shells. Shells are durable, and relatively easy to transport, preserve and examine. Thus, bivalve evolution has often been discussed in terms of conchological diversity adapting to environmental diversity (e.g. Stanley, 1970, 1977a, 1981).

But how often do conchological similarities reflect genetic versus adaptive similarities? How large a role does adaptive parallelism play in evolution? Such questions have led to the development of evolutionary systematics, a school that argues that systematics should be based on genetic, not morphological similarities. Thus, systematics is intended to reflect the evolutionary processes of species and higher taxa, and not merely the groupings of morphologically similar objects (De Queiroz, 1988; Lindberg, 1989).

Biomolecular techniques have provided some insight into adaptive parallelism, and resulting phylogenies of various extant groups of animals (e.g. Sibley and Ahlquist, 1991; Lowenstein, 1985; Jope, 1980) suggest that such parallelism is more prevalent than previously suspected. Parallelism was an important component in the early evolution of Bivalvia (Stanley, 1974); apparent cases occur frequently throughout Cardiidae (Savazzi, 1985) and Veneridae. More knowledge about the nature of the process might be gained by specifically examining how and where parallel evolution actually occurs throughout the evolution of a large successful family of organisms.

There are inherent limits in examining adaptive parallelism. Both "parallel" and "convergent" have been used to describe the acquiring of superficially similar characters through evolutionary time as similar adaptations to similar selective pressures by genetically distant taxa. When interpreting data on clusters of extant taxa, such a phenomenon can only be inferred, because data on ancestral species are absent. Depending on the definition of the points of reference, the inference can be wrong. The superficially similar but genetically distant taxa of A and B, for example, could appear to be convergent. Yet the immediate ancestor of A could be much more similar to that of B, so that A and B are really diverging currently, even if their overall history is one of convergence. Thus, to advance beyond inferrence, both extinct and extant points of reference must be defined, as well as the scale and scope of characters that comprise the presumed parallelism. For most studies, such data are rarely available. To succeed, fossils of ancestral taxa are needed from which both morphological and genetic data can be extracted.

Opportunities to do this within the Bivalvia exist through the biomolecular technique radio-immuno-assay (RIA). RIA measures immunologically genetic distance between taxa by measuring how much antibodies made against proteins of one species bind with proteins from another. The method is comparable roughly to DNA hybridization in that the products derived from the DNA, the proteins, are essentially hybridized via the mediation of the immunological reaction. The immunological distances resulting from RIA correlate highly with DNA hybridization (Sibley and Ahlquist, 1991), microcomplement fixation (Lowenstein *et al.*, 1981)

and DNA sequencing results (Lowenstein and Scheuenstuhl, 1991). RIA can detect extremely small amounts of protein (Luft and Yalow, 1974) and can distinguish closely related species (Lowenstein *et al.*, 1981; Lowenstein and Ryder, 1985) or compare widely divergent groups (Lowenstein, 1981). A unique feature of RIA is that it can provide information on proteins preserved in fossils as old as 60 million years (Westbroek *et al.*, 1979) and in calcareous matrices (Lowenstein, 1981; Lowenstein *et al.*, 1982, 1991; Molleson, 1982; Rainey *et al.*, 1984; Collins *et al.*, 1988). RIA has been used successfully on such disparate groups as algae (Olsen-Stojkovich *et al.*, 1986), gymnosperms (Price *et al.*, 1987), brachiopods (J. M. Lowenstein, pers. comm.), and reptiles, birds, and mammals (Lowenstein, 1981).

Within the Bivalvia, the Veneridae (Heterodonta: Veneracea) is an example of a large, global, diverse family with a rich fossil record; 500 or more extant species are classified into approximately 12 subfamilies, with 50 extant and 55 extinct genera, and 150 extant and 99 extinct subgenera. The earliest venerid fossils are approximately 130 million years old. Present in a wide variety of marine ecosystems, members of the family are characterized by having three cardinal teeth in each valve, and sometimes up to three anterior teeth (one in the left valve and two smaller interlocking ones in the right). A lunule, escutcheon, and pallial sinus are usually present; valves have concentric sculpture ranging from smooth to pronounced, and sometimes radial and divaricate sculpture, as well.

Venerid taxonomy is controversial, with several discrepancies among recent systematic works (Fischer-Piette and Delmas, 1967; Keen, 1969; Fischer-Piette, 1975; Fischer-Piette and Vukadinovic, 1975, 1977). No consistent, comprehensive conchological descriptions exist for the subfamilies, genera and subgenera. Genera, especially of minute clams, continue to be moved among subfamilies or changed by workers (Bernard, 1982; Lindberg, 1989), who must weigh which characters indicate true phylogenetic alliance versus parallel adaptations.

Little genetic information exists on the Veneridae. RNA sequencing data exist on three taxa (Bowman, 1989) but the amount is inadequate to draw significant conclusions about the family. Phylogenetic estimations are mostly based on conchological characters (e.g. Parker, 1949; Casey, 1952; Fischer-Piette and Vukadinovic, 1977) or shell microstructure (Shimamoto, 1986). The taxonomic confusion, lack of genetic information, and the size, age and diversity of Veneridae all highlight the potential gains to be had through RIA in understanding the evolution and systematics of the family.

Geographic and anatomical data indicate parallel evolution within Veneridae. Jones (1979) anatomically examined four species of the sub-family Chioninae: the west Pacific *Chione (Austrovenus) stutchburii* (Wood), the west Atlantic *Chione cancellata* (Linneaus) and *Mercenaria mercenaria* (Linneaus), and the east Pacific *Chione californiensis* (Broderip). The genus *Chione* is characterized by large, well-spaced concentric cords or lamellae, and strong radial ribs on the valves. *Mercenaria* has fine, closely spaced concentric threads that merge medially into smooth areas but can develop into weak, low lamellae laterally on the valves; a prominent, rugosely sculpted nymph is also present (Figs. 1f-g). Jones (1979) observed that anatomically, the three North American species (the western Atlantic Ocean and eastern Pacific Ocean) were allied much more closely to each other than to *C. stutchburii*, despite the conchological differences, indicating either a parallelism in anatomy among the American chionines, or in conchology between *C. stutchburii* and the American *Chione* spp.

Similarly, Keen (1969) places two geographically disparate but conchologically similar species, *Anomalocardia brasiliana* (Linneaus) and *Cryptonomella producta* (Anton) within the chionine genus *Anomalocardia* Schumacher, which she characterized as thick shells, with undulating concentric folds crossed by radial riblets, and large, impressed lunules. Harte (1992) observed similarities in sculpture, profile and nymphs between *Anomalocardia s. s.* and species of *Chione* and *Mercenaria*, however, and *A. brasiliana* overlaps geographically with *Chione cancellata*, which might indicate a common genetic origin. Past workers have occasionally classified *Anomalocardia* as a subgenus of *Chione* (Olsson, 1932; Parker, 1949).

As a preliminary test of RIA on venerid shells, I used RIA to determine whether the above two cases inferred conchological parallelism. The results are presented here.

MATERIALS AND METHODS

RIA data were obtained for two groups of taxa (Fig. 1) to test for parallel evolution. Nomenclature follows that of Keen (1969). The first group included: *Chione cancellata* (CC), *C. (Austrovenus) stutchburii* (CS) and *Mercenaria mercenaria* (Linneaus) (MM). Another west Pacific chionine species, *Timoclea (Glycydonta) marica* Linneaus) (TM) was also included. The second test group included *Anomalocardia brasiliana* (AB) and *A. (Cryptonemella) producta* (PR), which were compared with the first group. *Macrocallista nimbosa* (Lightfoot) (NI) (Veneridae: Pitarinae), was used as an outgroup to the two test groups of chionines.

RIA analyses on the above species were carried out under the direction of Dr. Jerold Lowenstein at the Medical Center of the University of California at San Francisco, where he has refined the method with over a decade of research on various groups of organisms. For each species, approximately 10 g of shell was ground to a powder and placed in 100 cc of 0.2M EDTA, a calcium chelating agent, for two days to dissolve the $CaCo_3$. The resulting protein solution was then

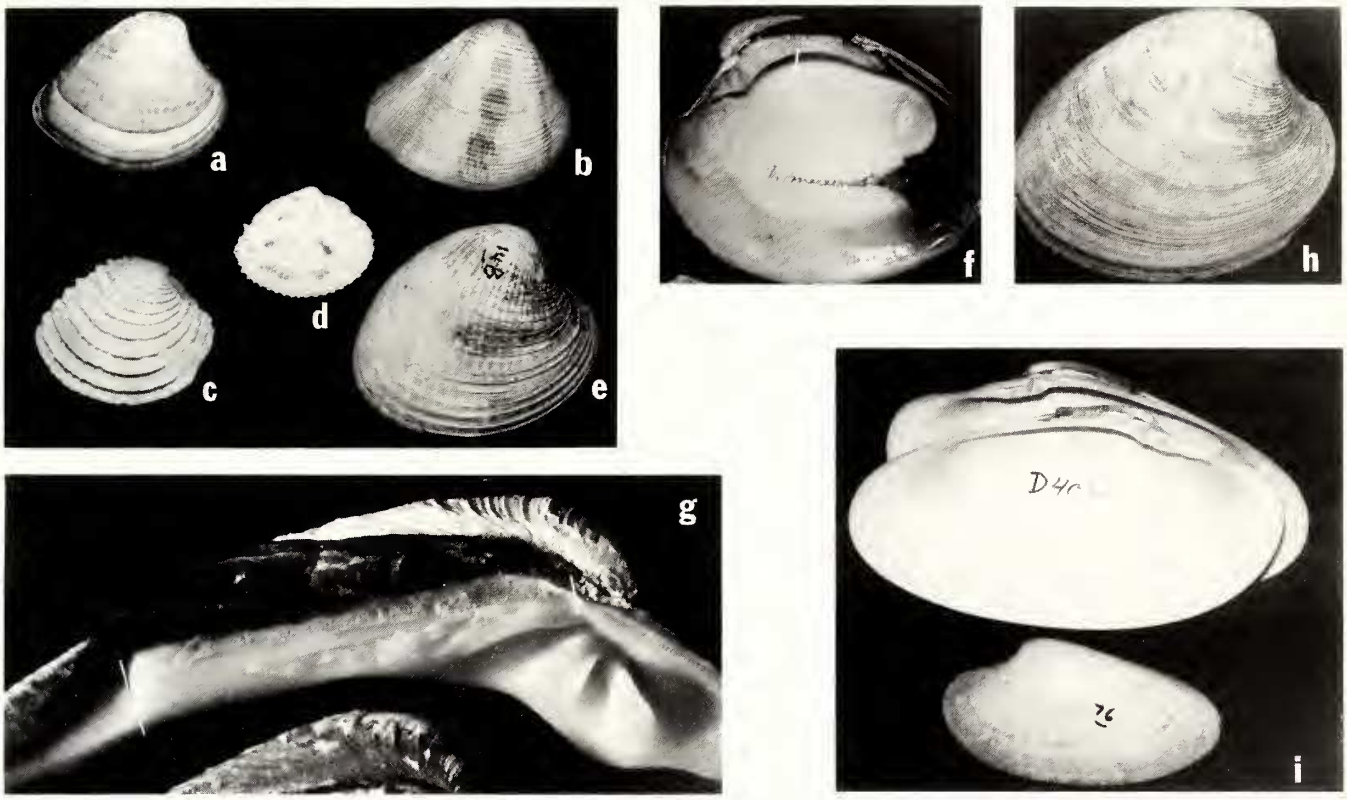

Fig. 1. a. *Anomalocardia brasiliana,* [shell length (s.l.) = 3.0 cm]; **b.** *A. (Cryptonemella) producta* (s.l. = 3.2 cm); **c.** *Chione cancellata* (s.l. = 2.8 cm); **d.** *Timoclea marica* (s.l. = 2.2. cm); **e.** *C. (Austrovenus) stutchburii* (s.l. = 3.8 cm); **f-h.** *Mercenaria mercenaria.* **f.** interior with rugose nymph indicated by white bar; **g.** rugose nymph (section between white bars = 2.2 cm); **h.** exterior (s.l. = 6.8 cm); **i.** *Macrocallista nimbosa* (s.l. = 8.6 cm, top; 5.5 cm, bottom).

tested for adequate reactivity against a pre-existing antibody made against *Arctica islandica* Linne. If the test was successful, a rabbit was injected at two points along its neck with 2.0 cc total of equal parts shell protein solution (antigens), and Freund's adjuvant, an agent used to stimulate antibody response. Every two weeks thereafter, the rabbit was further injected with 1.0 cc of shell protein solution. At the end of two months, the rabbit was drained of its serum, containing the antibodies made against the shell proteins.

A 0.1 dilution of the antigens for each species was made and tested against the antibodies of all the species. Antigens of species A were placed in a cup of a plastic microtiter plate and allowed to bind with the plastic for one hour. The excess was removed, and the cup was coated with 0.5% soy serum for five minutes to bind with the remaining plastic not bound by antigens A'. Excess serum was removed, and the rabbit antibodies of species B were added to the cup to bind with antigens A' for two days. Excess antibodies were removed, and the cup was rinsed with soy serum again. Radioactively tagged Iodine-125 goat antibodies produced against rabbit gamma globulin (GARGG) were then added to the cup for one day to bind with the antibody B-antigen A' complexes present. The cup was then rinsed with water, dried, placed inside a radiation counter tube, and its radioactivity measured. Additionally, for each set of antibodies, control cups without antigens were treated as above to determine the levels on nonspecific binding of the antibodies to the soy rinse serum. The radioactive count of an empty radiation counter tube represented the radioactive standard, the level of background radioactivity.

The resulting matrix of raw data was adjusted by first subtracting the nonspecific binding levels of the controls from tests involving their respective antibodies. The matrix was then divided by the radioactive standard, and the resulting quotients were expressed as percentages. The immunodistance (ID) for each reaction was calculated as follows:

$$ID_{A'B} = 100 \log_{10} (A'A/A'B);$$
$$ID_{AB'} = 100 \log_{10} (B'B/B'A);$$
$$ID_{AB} = 1/2 (ID_{A'B} + ID_{AB'});$$

where A'A, for example, is the reaction of the antigens (A) with the antibodies (A') of species A. A Fitch-Margoliash unrooted tree of the taxa was calculated from the resulting lower diagonal ID matrix using the program PHYLIP 3.1.

RESULTS

Results of the reactivity tests indicated that protein content varied noticeably among individual shells from the same species and even from the same lot, with some shells often not having adequate amounts of protein. Unknown and differing concentrations of antigens and antibodies do not interfere with interpretation of the resulting immunological distances, however, because of the nature of the immunological reaction, the experimental design, and the immunological distance algorithm. In RIA, the immunological reaction is a two step process: a plastic substrate is coated with antigen, which is subsequently exposed to reaction by antibodies. Because the binding substrate for antigens has a high affinity and limited capacity for antigens, the first step is a saturation reaction that requires little dissolved protein (i.e. antigen) to saturate all sites. Thus, different protein concentrations still result in saturation of binding sites. (This explains the fact that in preliminary trials, some antigen solutions ultimately yielded functioning antibodies, despite previous reactivity tests indicating that these solutions had practically no proteins present). The binding of the antibodies to antigens is an equilibrium reaction, however, so the amount of binding *is* proportional to antibody concentration. For different antibody concentrations, this is compensated by the counter reactions, which are incorporated *as proportions* into the immunological distance algorithm. Consider the example of antigens of species A reacting against two concentrations of antibodies from species B: weak (B'B = 20, B'A = 4), and strong (B'B = 40, B'A = 8). The resulting $ID_{A'B}$ remains unchanged because, as explained above, the concentrations of antigens A and B do not affect their reactions with the antibody, A'. The resulting $ID_{AB'}$ also remains unchanged, whether the strong or weak antibodies are used, because the B'B/B'A term is equivalent in both cases (20/4 = 40/8 = 5). Thus, ID_{AB} remains unchanged, regardless of the concentration of antibody used.

While shells recently separated from their animals (i.e., within days) exhibited adequate amounts of protein, there were no other clearcut predictors of adequate protein levels. Beachworn specimens often did not have adequate amounts of protein. Adequate protein solutions of all the above species were obtained, however, and antibodies were made against them.

Results are presented in Table 1 and figure 2. The matrix indicates that a deep division exists between Chioninae and Pitarinae, represented by NI, although NI is allied with the chionine taxa through TM, with the distance between NI and TM, 42, comparable to other distances among the chionine taxa (e.g. distances between TM and MM, and CS and MM). The unrooted Fitch-Margoliash tree groups the six chionine taxa in three pairs: AB and TM, CC and MM, PR and CS. All three pairs are conchologically dissimilar; the second pair are west Atlantic species and the third pair

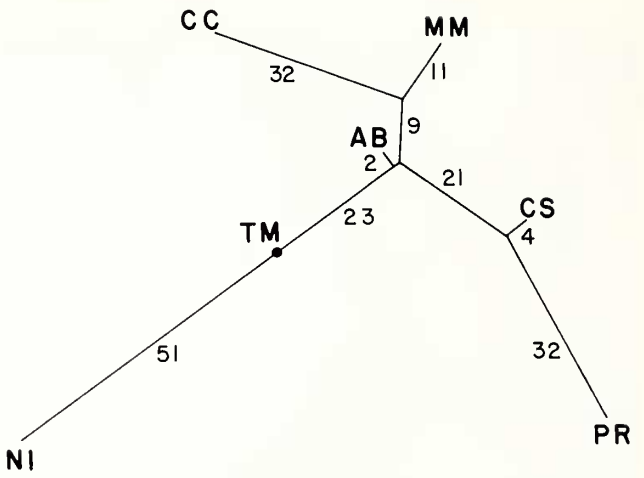

Fig. 2. An uprooted Fitch-Margoliash tree derived from Immunological Distance data on venerid taxa (abbreviations in text) (S. D. = 17.3, S. S. = 1.2).

are west Pacific species. Using CC as a point of reference, the tree indicates parallel evolution of either the cancellate sculpture of Chione twice in CS and TM or parallel evolution of the rostrate posterior and primarily concentric sculpture of *Anomalocardia* once in PR. Because no data are available on ancestral species, neither possibility assumes priority or an arbitrary direction. The matrix indicates that AB and TM are most closely allied (25), followed by PR and CS (39), and CM and MM (43). The matrix data agree well with the anatomical analyses of Jones (1979).

DISCUSSION

Much has been observed about bivalve adaptations (e.g. Carter, 1968; Stanley, 1970; Seilacher, 1974; Savazzi, 1985). The burrowing paradigm of Seilacher (1974) required that valve sculpture be perpendicular to the direction of burrowing, asymmetrical in crossection, and reduced medially (perimeter smoothening). Later experimentation and observations have supported this paradigm (Stanley, 1977b; Stanley, 1981; Savazzi, 1985). A clam burrows anteriorly, and assumes a life position with the posterior closest to the sediment surface. From this it is logical to assume that the anterior will facilitate burrowing and anchorage. The posterior, especially of shallow burrowers, coming into contact with the substratum only towards the end of burrowing, can contribute little towards it, and in cardiids posterior sculpture often does not conform to the burrowing paradigm (Savazzi, 1985). Being the point closest to the surface and predators, however, the posterior probably functions more towards predatory defense and reducing surface scour of sediment around the shell, thereby preventing disinterment. These are useful perspectives for analyzing venerid adaptations.

Most chionine clams burrow sluggishly and shallow-

Table 1. Immunological distances among various venerid taxa.

		Chione stutchburii	*Timoclea marica*	*Mercenaria mercenaria*	*Chione cancellata*	*Anomalocardia brasiliana*	*Anamalocardia producta*	*Macrocallista nimbosa*
Group 1	*Chione stutchburii*							
	Timoclea marica	55						
	Mercenaria mercenaria	46	43					
	Chione cancellata	59	106	34				
Group 2	*Anomalocardia brasiliana*	28	25	27	39			
	A. producta	37	77	77	103	56		
Outgroups	*Macrocallista nimbosa*	128	42	134	163	87	112	

ly, with the posterior tip positioned within 1 cm of the sediment surface (Stanley, 1970). The shells are moderately thick, prosogyrous, and subovate with a slightly angular posterior; most have strong valve ornamentation. In each species, the unique set of variations among these characters reflects a unique balance and compromise of adaptive strategies. This is illustrated in the above results, which not only indicate conchological parallelisms among west Pacific and North American chionine clams, but an extensive conchological diversification between closely related species.

The latter is especially well illustrated between *Mercenaria mercenaria* and *Chione cancellata*. *M. mercenaria* is a large, thick shelled, moderately rapid burrower (Stanley, 1970) of subdued, predominantly concentric sculpture. Such sculpture aids burrowing, while size and thickness help keep it anchored in the sediment (Kauffman, 1969). The clam adjusts burrowing depth (1-2 cm between posterior and sediment surface) and life position to sediment type, and inhabits an unusually broad range of environmental conditions (Stanley, 1970); this ability to adapt to sediment changes probably accounts at least partly for its wide exploitation of habitats. In contrast, *C. cancellata* is a small, thick shelled, slow burrower (Stanley, 1970) with well spaced, sharp, concentric lamellae, slightly corrugated from underlying, well spaced radial ribs. Its life position is with the posterior near or at the surface, and its habitat is comparatively restricted (Stanley, 1970). Stanley (1981) showed that the strong sculpture inhibited burrowing. Furthermore, a rough comparison of the ornamentation and burrowing rate indices of these and other Carribean *Chione* species (Stanley, 1970) indicates that burrowing rate is inversely correlated to the development of the concentric sculpture, a relationship supported on a broader scale throughout bivalvia (Kauffman, 1969). The strong sculpture of *C. cancellata* compensates posteriorly by reducing scour, however (Stanley, 1981); once interred, the sharp lamellae probably aid anchorage and discourage nipping of the short siphons or firm grippage by predators. Radial ribs not only confer rigidity to the shell (Kauffman, 1969) but in this case evidently strengthen the lamellae basally and the resulting corrugations of the lamellae (Stanley, 1981).

Lack of life history data precludes comprehensive comparisons of conchological features and life strategies for the pair of west Pacific chionines, *Chione stutchburii* and *Anomalocardia producta*. While they have different profiles and concentric sculpture, they have similar radial patterns. Radial ribs are virtually absent anteriorly, appearing faintly medially and predominating the posterior sculpture. Posterior radial ribbing offers an acceptable approximation to being perpendicularly oriented to water currents, thereby reducing surface scour (Savazzi, 1985), which might explain its presence in both species, although not as a parallel adaptation but as a commonly derived one. The concentric sculpture in both species is more subdued than that of *C. cancellata*, indicating that it functions in them more as a burrowing aid than anchor. For the more streamlined species, *C. stutchburii*, it could enable more rapid burrowing, enabling exploitation of less stable habitats; indeed, the species inhabits a wide variety of sediments, ranging from less stable sediments of sand and gravel to a more stable muddy sand within estuaries and enclosed bays (Beu and Maxwell, 1990). For *A. producta*, concentric sculpture might compensate at least partly for its lack of streamlining.

The third linked pair of chionines, *Timoclea marica* and *Anomalocardia brasiliana*, are the most closely linked of the pairs immunologically, yet exhibit wide disparities in profile, sculpture and geography. Their different adaptive pathways exhibit interesting parallels. Both live near the surface, are similar in size and probably equally slow infaunal burrowers. *T. marica* has sculpture closely similar to *Chione cancellata*: well developed lamellae (though not as widely spaced) that flare posteriorly, corrugated by well separated radial ribs. A plausible inferrence is that it functions similarly, inhibiting burrowing but serving as an anchor, an anti-scour mechanism posteriorly, and to discourage predators.

Similar functions in *Anomalocardia brasiliana* probably are accomplished through its unusual profile rather than its relatively subdued sculpture, which aids burrowing. *A. brasiliana* and *Chione cancellata* are equally slower burrowers (Stanley, 1970); the blunt, relatively obese anterior profile probably inhibits burrowing. The compensations, however, are several. Angular to rostrate posteriors serve to

elevate the siphonal flow with a minimum of shell excretion while permitting the center of gravity to remain relatively deep, although streamlining is sacrificed (Stanley, 1970). The rostrate posterior maximizes this effect. The life position in the sediment is perpendicular to the sediment surface (Stanley, 1970), and this minimizes the posterior surface vulnerable to scouring and predators. Such advantages could result in parallel selection for this trait, and, indeed, this trait appears in several subfamilies of Veneridae [e.g. *Lepidocardia floridella* (Gray), Pitarinae; *Eumarcia paupercula* (Holten), Tapetinae; *Timoclea malonei* (Vanatta) and *T. peresi* Fischer-Piette and Vukadinovic, Chioninae].

Another difference between *Timoclea marica* and *Anomalocardia producta* is that the posterior dorsal margins of *T. marica* are finely crenulated, while those of *A. producta* slightly interlock by means of a long, shallow fold in the right valve. Hypotheses on defensive functions of crenulated margins include increasing resistance of the shell to compression from shell-crushing predators (Waller, 1969), restricting predatory access of starfish, and creating a tight seal (Carter, 1968), thereby preventing release of diagnostic chemicals into the environment, and increasing survival times in a predator's digestive tract (Vermeij, 1987). Restricting predatory access and creating a tight seal might be effected equally by marginal folding, and function similarly. Jones (1979) observed, for example, that marginal folding effectively keeps the posterior dorsal margin closed while siphons are extended, and suggests that the resulting marginal overlaps might deter polydorid polychaete pests. Additionally, both marginal interdigitation and folding can thicken the marginal juncture, discouraging boring predators. Which adaptation is ultimately chosen might depend ultimately on slight differences in ontogeny and environment.

The data indicate, then, that *Timoclea marica* and *Anomalocardia brasiliana* are closely related species exhibiting different sculpture, profiles and posterior margins that function similarly in aiding anchorage and discouraging predators. In both species, efficiency in burrowing is sacrificed for advantages in anchorage and predatory discouragement.

The Fitch-Margoliash tree indicated parallel evolution of either rostrate posteriors and their accompanying characteristics, or cancellate sculpture. Arguments for the former are advanced, above. Parallel adaptation of the cancellate sculpture characteristic of *Chione* and *Timoclea* could occur in various species because of the cumulative advantages offered by ribbing, and the ontogenetic ease with which strong, well spaced concentric sculpture can be modified into structures that aid anchorage and defense (lamellae) or burrowing (cords or ridges), facilitating evolution into different life strategies. Indeed, this transition can be seen within several species of venerids, such as *Mercenaria mercenaria*, where juveniles, more vulnerable to disinterment, have widely spaced low, anchoring, concentric lamellae (Pratt and Campbell, 1956) that gradually become closely spaced, more subdued threads, medially worn smooth in adults.

The RIA data have several ramifications for venerid systematics, which, for the above chionines, is beset with definitional problems. As developed by Keen (1969), the genus *Anomalocardia* is paradoxical: except for *Anomalocardia s. s.*, the concentric sculpture of the included subgenera are concentric cords, not the undulating folds given in the generic definition (Fig. 1a-b). The result is a de facto assemblage of chionine species with rostrate posteriors and primarily concentric sculpture. Immunological distances indicate the features are a parallel adaptation, although the tree allows an interpretation of them as a derived adaptation.

Besides having different concentric sculpture, *Anomalocardia producta* differs from *A. brasiliana* in lacking a rugose nymph and crenulated margins, and in having pronounced posterior radial ribs. While not as obvious a trait as a rostrate posterior, the rugose nymph of *A. brasiliana* is a distinct marker for linking the taxon conchologically to immunological allies, such as *Mercenaria*.

Matrix data support the morphological transition proposed by Harte (1992) linking *Anomalocardia s. s.* to *Mercenaria* and two subgenera of *Chione, Lirophora* and *Iliochione*, and *Mercenaria*. Besides a rugose nymph, present at various degrees in all four taxa, specific traits include concentric undulations as the predominant sculpture, with a posterior that ranges from angular *(Mercenaria)* to rostrate (*Iliochione*, some *Lirophora* and *Anomalocardia*).

Several systematic changes are indicated by the above data. Matrix data indicate that *Cryptonomella* is more closely related to *Chione (Austrovenus) stutchburii* than *Anomalocardia*; it should stand as a separate genus until it can be demonstrated to be closely linked to a senior genus. *C. (A.) stutchburii* is more closely allied to *Anomalocardia* and *Mercenaria* than to *Chione* and should stand as a separate genus until its intergeneric relationships are further clarified. The relationships of the other subgenera of *Chione* (*Chionista* Keen, 1958; *Chionopsis* Olsson, 1932; *Panchione* Olsson, 1964 and *Securella* Parker, 1949) should be clarified before deciding the systematic relationships of *Chione* to other chionine taxa.

The immunological distances between *Macrocallista nimbosa* and most of the chionine taxa indicate a deep division within the family, an observation of systematic workers, as well. With some exceptions, venerid taxa fall roughly into two groups: 1) those with well developed anterior lateral teeth, and simple valve sculpture (Pitarinae, *et al.*); 2) those with little or no anterior lateral teeth, and often strong valve sculpture (Chioninae, *et al.*). Although venerid classification has been in a state of constant flux, some past workers have tended to sometimes merge existing subfamilies within these

two groups (Fischer, 1887; Dall, 1902; Jukes-Browne, 1914). In contrast, Frizzell (1936) elevated the ten subfamilies that existed by then into separate families. Keen (1969) returned them to subfamilies, and the relatively close alliance of *M. nimbosa* to *Timoclea* indicates that the family is monophyletic.

CONCLUSIONS

Radio-immuno-assay has been used successfully in conjunction with conchological and anatomical analyses to help illustrate various evolutionary processes within Veneridae. RIA and conchological analyses of Pacific and Atlantic Ocean chionines indicate that between distantly related species there exists one or more strong conchological parallelisms, which have as their bases adaptations towards predatory defense, positional stability, and burrowing efficiency.

RIA analyses also indicate that closely related species have undergone extensive conchological diversification. This diversification probably results from adaptation to different lifestyles, or utilizing different adaptations for similar functions. The conchological differences between *Mercenaria mercenaria* and *Chione cancellata* reflect different life strategies for predatory defense and positional stability. *M. mercenaria* appears to rely on burrowing abilities, size and thickness, while *C. cancellata* relies on sculpture, thickness, and a more specialized habitat. Conchological differences between *Timoclea marica* and *Anomalocardia brasiliana*, in contrast, reflect different conchological strategies that effect parallel adaptive compromises, and cope with parallel lifestyles.

Ramifications for systematics include separating *Cryptonomella* from *Anomalocardia*, separating *Austrovenus* from *Chione*, and reassessing the systematic relationships of *Anomalocardia, Timoclea, Mercenaria* and *Chione*. A deep division exists between the subfamilies Pitarinae and Chioninae, although a single, relatively close link between them indicates that the family is monophyletic. Geographic and anatomical data, and obscure conchological traits can sometimes indicate genetic alliances, and should be utilized in assessing systematic relationships.

RIA has the potential for proving examples of parallel evolution between extant bivalve taxa and their ancestors. Further research utilizing fossil taxa is planned.

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